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Biopesticidal effects of some Medicinal Plant extracts on Growth Parameters and Control of Diseases in S*olanum melongena* L.

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ABSTRACT:

The present study was undertaken to determine the effect of some medicinal plants on growth parameters and diseases, like wilt and leaf spot of egg plant. To understand the mechanism, the phytochemical analysis of plants and its effect on bacterial and fungal cultures were investigated. The effects of selected plant extracts on eggplants were observed. Ten medicinal plants were applied for the control of diseases, and its antibacterial and antifungal effect was tested against *Cercospora melongena*, *Fusarium oxysporum*, and *Psedumonas solancerum* that cause spot of leaves and wilt disease in *Solanum melogena*. Extract of *Z.jujube* were found significantly superior in inhibiting the bacterial growth of *Psedumonas solancerum* at 15% (4.50±0.09) and mycelial growth of *Fusarium oxysporum* and Cercospora melongena in culture medium at 15% of *V.negundu* (22.78±2.60) and 5% of *P.nigrum* (31.06±0.04). Among the plant extract tested in field, *A.marmelos* and *A.conyzoides* was found most effective in reducing the wilt disease by 53.36% and *P.nigrum* and *A.indica* reduced the leaf spot by 85.32% and 82.33% respectively in *Solanum melongena*. The results suggest that the application is growth promotive, cost effective, and non-hazardous in agro-ecosystem. Results on growth parameters indicated significant differences in the shoot length, number of leaves, number of branches, number of flowers, number of fruits, etc. in all treatments.

Keywords: Biopesticides, Plant Diseases, Growth Parameters, Plant Diseases, *Solanum melongena*

INTRODUCTION

Sustainable pest management is an essential prerequisite of farming in any country. Techniques employed for management and eradication of pests are always associated with economic risks and uncertainties. These risks are many times higher in developing countries than in industrialized countries [1]. Use of synthetic chemicals for controlling pests lead to problems, such as destruction of beneficial non-target organisms (parasitoids and predators), thereby affecting the food chain and thus, having a negative impact on the biological diversity. The injudicious use of synthetic pesticides can lead to secondary outbreaks of pests that are normally under natural control resulting in their rapid proliferation. Some prior studies have also reported cases of pests becoming tolerant to insecticides, resulting in increase in the application rates of the insecticides to two or even three times [2]. Due to other additional problems, such as health hazards, undesirable side effects, and environmental pollution caused by the continuous use of synthetic chemical pesticides [3], there is renewed interest in the application of herbal pesticides for crop protection. Scientists are now utilizing indigenous plant materials to protect insect infestation of Plants. The use of such plant extracts for controlling pests is not a new innovation, as it has been widely used by small-scale subsistence farmers for pest control in their farms as it could be produced easily by them. Use of locally available plants for controlling pests is an ancient technology that has been widely adopted in many parts of the world [4]. Most of the botanical pesticides used are non-selective poisons that target a broad range of pests. Botanical pesticides are

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biodegradable and can be used as a practical sustainable alternative for protecting crops [5]. They maintain the biological diversity of predators by reducing environmental contamination and human health hazards [6]. Research on active botanical pesticide ingredients, their preparation, application rates, and environmental impact are needed for sustainable agriculture [7]. With the increase in consumer demands for organic foods, research on botanical pesticides is also gaining momentum [3].

Solanum melongena (commonly known as brinjal or eggplant) is a solanaceous crop of sub-tropics and tropics. The Indian name brinjal is derived from Arabic and Sanskrit, whereas the name eggplant has been derived from the shape of the fruit of some varieties, which are white and resemble in shape to chicken eggs. Although it is a perennial crop, but is grown commercially as an annual crop. Varieties of eggplant display a wide range of fruit shapes and colors, ranging from oval or egg-shaped to long club-shaped, and from white, yellow, green, to different degrees of purple pigmentation to almost black. It develops into bushy plants with large, fuzzy leaves that grow to a height of about 60 to 120 centimeters. The plant is erect, compact, and well branched. It has a rather fibrous or lignified root system. The leaves are large, simple, lobed, and are located alternate on the stems. Eggplants are affected by various diseases, like wilt, blight, little leaf, and leaf spot causing heavy losses to the crop production. The fungi *Cercospora melongena* and *Fusarium oxysporum, and the bacteria Pseudomonas solancerum* cause wilting and leaf spot affecting its field production greatly. Leaf spots caused by Alternaria spp. are irregular in shape with concentric rings enlarge and cover the entire leaf blade. Early infections by Alternaria spp. may cause a seedling dieback known as “collar rot.” Cercospora sp. causes small, irregular leaf spots that later are covered with gray sporulation. Fruit lesions start as necrotic spots and develop into sunken, scab-like lesions that extend into the flesh of the fruit, turning it hard and brown. In order to prevent the plant from these diseases and from pathogens, chemical control methods are needed. Because of high cost of chemical pesticides and their hazardous consequences, the use of different biodegradable materials, like fresh plant extracts has gained importance during last three decades [6, 8, and 9]. In this context, the present study was undertaken in order to find out the effort of ten botanical plant extracts on *Solanum melongena* for controlling wilt and leaf spots.

MATERIALS AND METHODS

The medicinal plants selected for the current study were: *Pongamia piñata; Aegle Marmelos; Azadirachta Indica; Brassica Campestris; Piper Nigrum; Euphorbia tirucalli; Vitex Negundu; Ageratum Conyzoides; Tagetes Patula; and Zigiphus jujube.* The seeds of eggplant were sown on raised nursery beds of size 7.2 X 1.2 m and 10-15 cm in height to raise seedlings for transplanting into the field. Ten such beds were raised to grow seedlings for planting in one hectare area. Distance of about 70 cm was kept between two beds to carry out other mandatory operation like watering, weeding, etc. Raised beds were necessary so as to avoid the problem of water logging in heavy soils. In sandy soils, however, sowing can be done in flat beds. Fresh plant parts of 10 medicinal plants were collected from local regions of Odisha. The plant materials were washed thoroughly; air dried, and were then homogenized to fine powder and stored in airtight bottles.

Hundred grams from each of the dried, powdered plant sample were weighed and were placed in a 1000 ml flask. The flask was closed with cotton balls and was covered with aluminum foil. It was then filtered with the help of cheese cloth. 10ml solution of the liquid extract, which was placed in the flask, was sprayed on the leaves of 3 eggplants on a weekly basis. The following growth parameters were recorded on a weekly basis. Morphological measurements of the eggplant were taken at 7, 14, 21, 28, 35, 42, 49, 56, 63,

and 70 days post transplantation for determining changes in growth parameters. Morphological measurements of eggplant were taken at every 7 days interval after transplantation to determine the disease control rates. From the day of transplantation till harvest time the experimental fields were maintained and the following parameters of eggplants were recorded at every 7day interval: shoot height; number of leaves; number of branches; number of flowers; and number of fruits per plants. Fruits were collected, weighted, and were stored at 34°C for routine observation.

The growth rate of diseases in eggplants (case) and the ability of different plant extracts to control them (control) were analyzed in the current study. Diseases included wilt, blight, little leaf, leaf spot, etc.

PHYTOCHEMICAL SCREENING OF PLANT MATERIALS

Presence of saponins, tannins, carbohydrates, alkaloids, flavonoids, glycosides, steroids, proteins, and alkaloids were detected by simple qualitative [10] and quantitative methods [11].

ANTIMICROBIAL ASSAY OF TEST MICROORGANISM

Bacterial cultures of *Pseudomonas solancearum* that were used for antimicrobial assay of test organisms were obtained from the culture collection centre, Department of Microbiology, Orissa University of Agriculture and Technology, Odisha, India,. The bacteria were maintained on nutrient broth (NB) at 37^0C till required for analysis. The *in vitro* antimicrobial activity of the sample solution was done by disc diffusion method [12].

ANTIFUNGAL ASSAY OF TEST FUNGI

The infected portion of the plants were extracted and cultured on Sabouraud's dextrose agar and the plates were incubated at room temperature for two days. Grayish brown mycelia were seen which turned later to black color. Smears were prepared and stained with lacto phenol cotton blue stain and were observed under high power microscope. The agar dilution assay was carried out according to [13] with a slight modification. Thirty nine grams of potato dextrose agar (PDA) powder was boiled until the agar completely dissolved in 1 L of distilled water. The solution was then sterilized using autoclave at 121°C for 15 min. 19 ml of the sterilized PDA and 1 ml of plant extract were mixed and plated on the sterilized petridishes (8.5 mm in diameter). 10 mm diameter of mycelia discs were inoculated at the centre of the medium. The antifungal assay was divided into 10 different treatments as crude extract of leaves in different Concentration (5, 10, and 15%). Colony growth was determined on the basis of linear dimensions. The percent reduction (Rr) or stimulation (Rs) of colony diameter by each extract was determined using the following formula [14]:

$$Rr = \frac{(R1 - R2)X100}{R1} \quad \text{Equation 1}$$

$$Rs = \frac{(R2 - R1)X100}{R2} \quad \text{Equation 2}$$

Where, Rr = percent reduction in colony diameter; Rs = percent stimulation in colony diameter; R1 = colony diameter on the untreated medium (control); and R2 = colony diameter on the treated medium.

STATISTICAL ANALYSIS

The experimental data were statistically analyzed .The significance of differences between the treatments was evaluated by one way analysis of variance at the significance level of 95 % .The Statistical software SPSS version 12.0 was used in the analysis (SPSS INC.2003). All the data were analyzed with students 't' test .The value of growth parameter and disease data were statistically analyzed .In the test of significant *(P<0.05),**(P<0.01),***(P<0.001) indicate the treatments were significant at probability level respectively.

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Table-1: Effect of medicinal plant treatment on significance level of various morphological parameters in *Solanum melongena*

PLANT	Shootheight	Branches	Leaf no	flowers	Fruits
P.pinatta	*	NS	NS	NS	NS
A.marmelos	**	NS	NS	*	***
A.indica	***	NS	NS	NS	NS
B.campestris	NS	**	**	***	**
P.nigrum	***	NS	NS	NS	NS
E.tirucalli	***	NS	NS	**	**
V.negundu	***	NS	NS	NS	***
A.conyzoides	***	**	**	NS	**
T.patula	NS	**	**	*	**
Z.jujube	***	***	***	NS	***

Table-2: Effect of medicinal plant treatment on significance level of various diseases in *solanum melongena*

PLANT	***Wilt***	***Leafspot***
P.pinatta	*NS*	**
A.marmelos	**	**
A.indica	***	***
B.campestris	*NS*	*
P.nigrum	*NS*	***
E.tirucalli	*NS*	**
V.negundu	*NS*	**
A.conyzoides	***	*NS*
T.patula	*NS*	*NS*
Z.jujube	*NS*	**

NS-Not Significant

Table-3: Antibacterial and Antifungal activity of 10 medicinal plant extracts

Plant species	**Concentration (%)**	***Psedumonas solancerum***	***Cercospora melongena***	***Fusarium oxysporum***
P. pinatta	5	1.10±0.43	20.40±0.07	10.22±0.04
	10	1.30.±0.32	18.84±0.02	12.07±0.02
	15	1.40±0.09	12.22±0.04	16.06±0.02
A.marmelos	5	1.21±0.23	18.91±0.07	11.52±1.86
	10	1.50±0.06	16.56±0.04	12.12±1.78
	15	1.75±0.01	11.05±0.05	18.26±0.34
A.indica	5	1.24±0.22	12.62±0.06	18.20±1.00
	10	1.30±0.01	10.91±0.03	19.66±0.60
	15	1.70±0.21	8.86±0.03	20.42±0.57
B.campestris	5	1.40±0.09	18.21±0.10	12.51±0.78
	10	1.50±0.54	12.30±0.02	14.47±1.00
	15	1.90±0.16	10.20±0.03	16.08±0.04
P.nigrum	5	1.12±0.31	31.06±0.04	10.94±0.04
	10	1.30±0.09	25.05±0.11	11.84±0.04
	15	1.50±0.56	20.07±0.11	12.22±0.04
E.tirucalli	5	1.35±0.31	12.57±0.12	12.40±0.07
	10	1.50±0.15	16.35±0.06	14.84±0.02
	15	1.75±0.25	15.11±0.02	16.22±0.04
V.negudu	5	1.30±0.01	12.08±0.03	20.66±0.60
	10	1.70±0.21	19.06±0.04	21.42±0.57
	15	2.50±0.29	10.06±0.05	22.78±2.60
A.conyzoides	5	1.40±0.02	26.16±0.02	12.62±0.06
	10	1.45±0.09	21.00±0.04	13.91±0.03
	15	2.10±0.09	16.06±0.02	14.86±0.03
T.patula	5	2.25±0.09	26.28±0.10	10.21±0.10
	10	3.00±0.32	20.40±0.02	12.30±0.02
	15	3.50±0.05	12.17±0.03	14.20±0.03
Z.jujube	5	3.00±0.09	12.00±0.10	7.06±0.04
	10	4.00±0.41	18.59±0.11	9.05±0.11
	15	4.50±0.09	20.93±0.03	10.07±0.11

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Table-4: Qualitative Analysis of Phytochemicals of Medicinal Plants

S/n	Plant Species	Alkaloid	Saponins	Tannins	Glycosids	Anthar quinins	Terpens	Steroid	Flavonoid	Reducing sugar	Pentose	Carbohydrates	Proteins	Amino acids
1.	*Pongmia piñata*	–	+	–	–	–	–	+	+	–	–	–	–	–
2.	*Aegle marmelos*	–	+	–	+	–	+	+	+	+	–	+	+	+
3.	*Azadirachta indica*	+	+	+	+	–	+	+	+	+	+	+	–	–
4.	*Brassica campestris*	–	–	+	–	–	–	–	+	–	–	–	+	–
5.	*Piper nigrum*	+	_	+	+	+	+	+	+	+	_	_	_	-
6.	*Euphorbia tirucalli*	+	+	+	+	–	–	+	–	–	–	–	–	–
7.	*Vitex nigundu*	+	+	+	+	+	+	_	_	_	_	_	_	_
8.	*Ageratum conyzoides*	+	_	_	_	+	_	+	+	_	_	_	_	_
9.	*Tagetes patula*	+	_	_	_	+	_	+	+	_	_	_	_	_
10.	*Zighiphus jujube*	+	+	+	_	_	_	+	+	+	_	_	_	_

(+) PRESENT, (-) ABSENT

Table-5: Quantitative Analysis of Phytochemicals of Medicinal Plants

S/n	Plant Species	Alkaloids (%)	Tannin (%)	Saponin (%)	Flavonoids (%)
1.	*Pongmia pinatta*	ND	15.75±0.04	0.87±0.01	3.06±0.03
2.	*Aegle marmelos*	1.036±0.02	14.16±0.12	3.83±0.02	0.94±0.00
3.	*Azadirachta indica*	1.13±0.01	6.13±0.08	0.21±0.01	2.09±0.10
4.	*Brassica campestris*	0.90±0.04	12.33±0.18	ND	4.53±70.10
5.	*Piper nigrum*	1.11±0.04	10.2±0.11	ND	4.8±0.05
6.	*Euphorbia tirucalli*	0.75±0.01	11.2±0.11	0.15±0.00	ND
7.	*Vitex nigundu*	0.86±0.00	9.39±0.08	3.03±0.08	5.10±0.63
8.	*Ageratum conyzoides*	10.2±0.11	ND	ND	4.8±0.05
9.	*Tagetes patula*	1.53±0.01	ND	ND	0.2±0.005
10.	*Zighiphus jujube*	0.49±0.01	0.65±0.02	8.08±0.05	0.59±0.00

ND-Not detected

Table 6: % reduction of diseases in eggplants by 10 medicinal plant

Treated plant	*W*	*LS*
P.pinatta	51.92%	55.98%
A.marmelos	53.36%	75.44%
A.indica	41.34%	82.33%
B.campestris	25.48%	60.47%
P.nigrum	9.13%	85.32%
E.tirucalli	32.69%	75.59%
V.negundu	12.01%	63.77%
A.conyzoides	53.36%	50.59%
T.patula	48.07%	56.43%
Z.jujube	38.94%	63.32%

(W-wilt, LS-Leafspot)

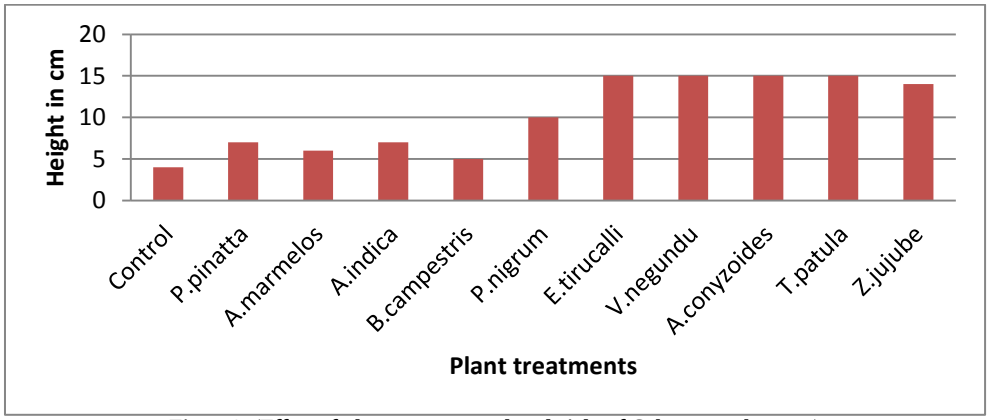

Figure1: (Effect of plant extract on plant height of *Solanum melongena*)

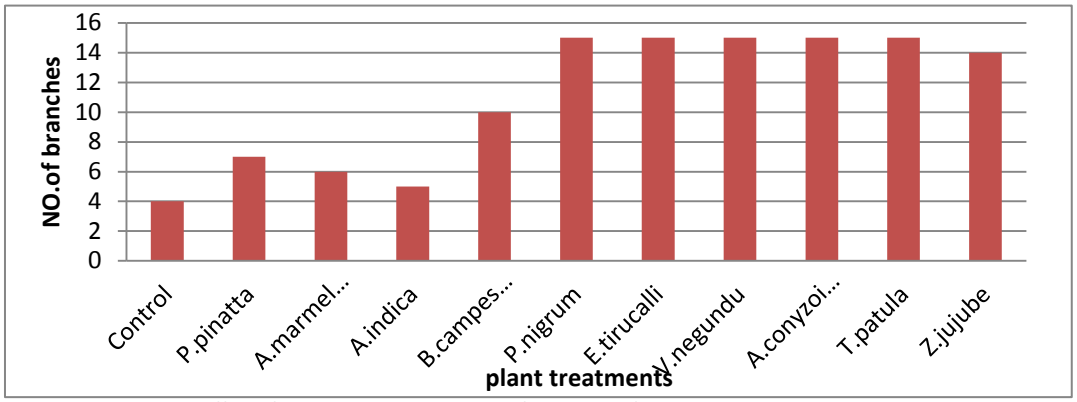

Figure2: (Effect of plant extract on number of branches of *Solanum melongena*)

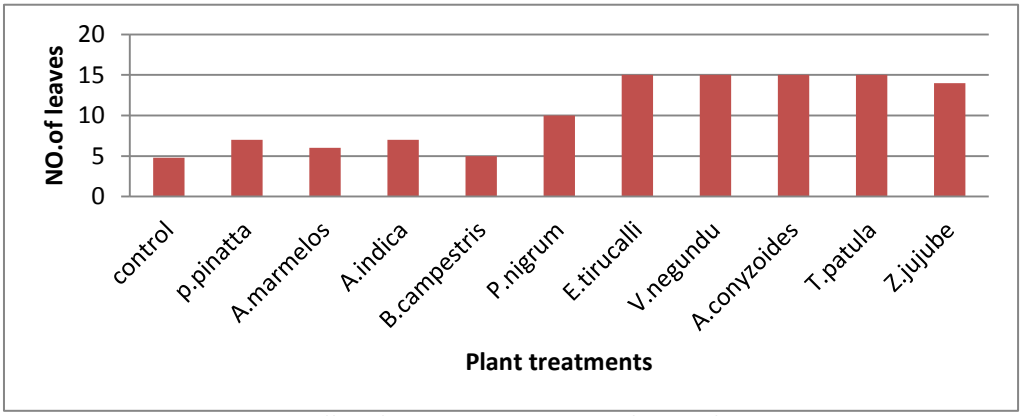

Figure 3: (Effect of plant extract on number of leaves of *Solanum melongena*)

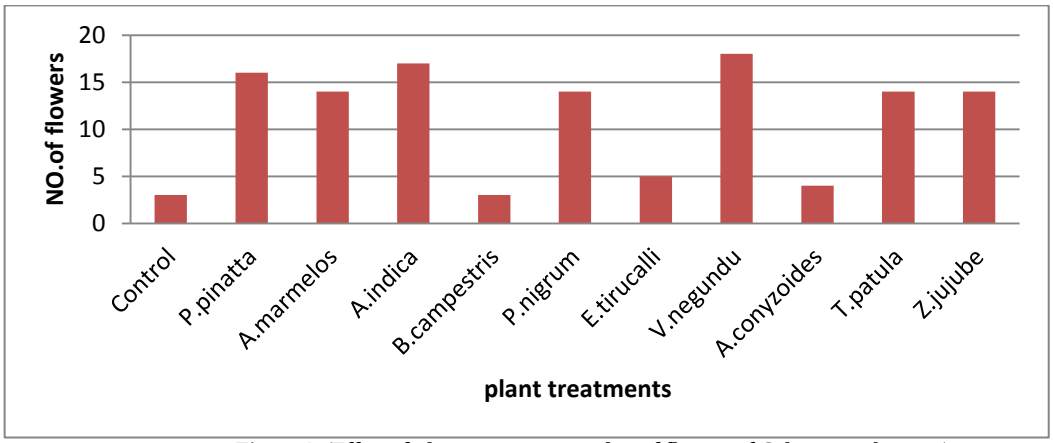

Figure 4: (Effect of plant extract on number of flowers of *Solanum melongena*)

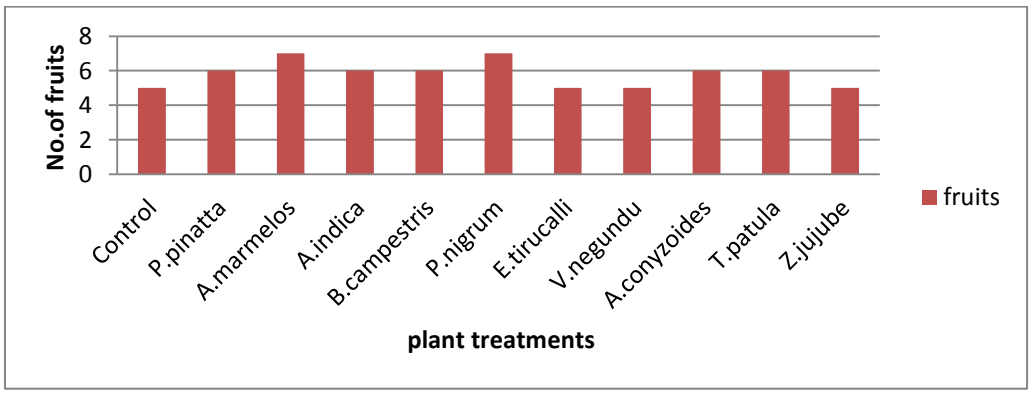

Figure5: (Effect of plant extract on number of fruits of *Solanum melongena*)

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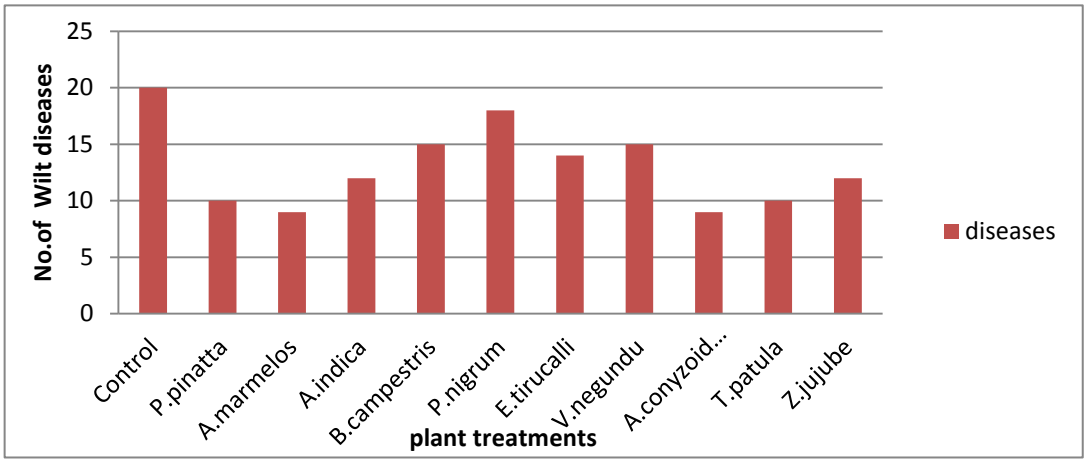

FFiguFigure 6: (Effect of plant extract on number of Wilt diseases of *Solanum melongena*

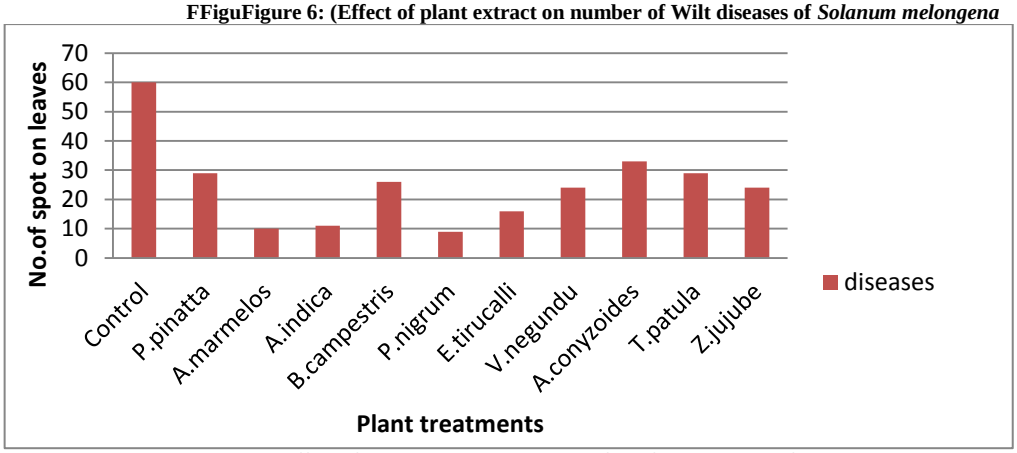

Figure7: (Effect of plant extract on number of Leafspot diseases of *Solanum melongena*)

RESULTS AND DISCUSSION

EFFECT OF MEDICINAL PLANTS ON GROWTH PARAMETER

The data presented in Table-1 and Figures-1, 2, 3, 4, and 5 in shoot height, number of branches, number of leaves, number of flowers, number of fruits in brinjal was noticed. Plant height increased significantly at (P<0.001) in *A.indica, P.nigrum, E.tirucalli, V.negundu, A.conyzoides, and Z.jujube extracts.(Table-1,Fig-1).* The number of branches were significantly increased at (P<0.001) in *Z.jujube and at(P<0.01)in B.campestris, A.conyzoides, and T.patula extract* (Fig-2). The number of leaves were significantly increased at (P<0.001) with treatment of *Z.jujube* extract *and at(p<0.01) in B.campestris, A.conyzoides, and T.patula extracts* (Fig-3). The number of flowers were significantly increased at (P<0.001) in *B.campestris* and at (P<0.01) in *E.tirucalli* and at (P<0.05) in *A.marmelos and T.patula* extract (Fig- 4). The number of fruits were significantly increased at (P<0.001) in *A.marmelos, V.negundu, and Z.jujube* extract and at (P<0.01) in *B.campestris, E.tirucalli, A.conyzoides, and T.patula* extract (Fig-5).

EFFECT OF MEDICINAL PLANTS ON WILT AND LEAF SPOT

10 medicinal plants were screened in the field for their efficacy against wilt in egg plant. Results indicated a significance reduction in wilt in *A.indica and A.conyzoides* treatment (P<0.001) followed by *A.marmelos* treatment (P≥0.01), while rest of the plant extracts were not effective in inhibiting the plant diseases (Table-2,Fig-7). Results indicated that there was a significance reduction in leaf spot in *A.indica and P.nigrum* treatment (P<0.001) (Table-2,Fig-8),

followed by *P.pinatta, A.marmelos, E.tirucalli ,V.negundu and Z.jujube treatment* (P<0.01), while rest of the plant extracts were not effective in inhibiting the plant diseases.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

The antimicrobial effect of crude medicinal plant extracts of 10 plants species were determined by in vitro studies using water as a solvent. Solvent extracts of *P.pinatta, A.marmelos, A.indica, B.campestris, P.nigrum, E.tirucalli, V.negundu, A.conyzoides, T.patula, and Z. jujube* at concentrations of 5, 10, and 15 exhibited the zone of inhibition. In case of *Psedomonas solancerum* the higher inhibition was noticed in *Z.jujube* extract significantly at 15% (4.50±0.09). Except *Z.jujube*, rest medicinal plants showed moderate inhibition in all concentrations followed by *A.marmelos,V.negundu, E.tirucalli, T.patula, A.indica, P.pinatta, B.campestris, and P.nigrum* (Table-3). All the medicinal plant extracts *P.pinatta, A.marmelos, A.indica, B.campestris, P.nigrum, E .tirucalli, V.negundu, A.conyzoides, T.patula, and Z.jujube* at 5,10,and 15% inhibited different degrees of mycelial growth. In case of *Cercospora melongena, P.nigrum* showed high inhibition of mycelial growth at 5% (31.06±0.04) and in case of *Fusarium oxysporum, V.negundu* showed high inhibition of mycelial growth at 15% (22.78±2.60).

The inhibitory effect of the plant extract might be attributed to the presence of secondary metabolites of medicinal plants (Table-4 and 5). Greater inhibitions of fungal growth were observed by P.nigrum for *Cercospora melongena* and *V. negundu* for *Fusarium oxysporum*. However, lower concentration of the extract supported the average mycelia growth inhibition. *Fusarium* and *Cercospora* are the common soil inhibiting plant pathogenic fungus which causes diseases such as wilt and leaf spot in eggplant.

Several other species of this genus are responsible for huge loses to their respective host crop. Natural chemicals and their use for integrated plant protection is one of the focuses of research workers all over the world .These results of the present investigation are clear indication for the potential of plant extracts to control fungal pathogens and these compounds can be used.

It is evident of the result that all the plant extracts inhibited bacterial growth. Antibacterial test by using a simple MIC test was done. Among the plant extract of *Z.jujube* showed higher antibacterial activity where as *P.pinatta* showed lower antibacterial activity .Successful attempts have been made for the management of *Fusarium oxysporum*, *Cercospora melongena* and *Psedomonas solancerum*. The plants were examined for disease symptoms and a quantitative assessment. In general disease incident was reduced by the application of plant extract.

Plant extract of *A.marmelos* and *A.conyzoides* significantly reduced (53.36%) leaf infections (wilt). Similarly *P.nigrum* (85.32%) and *A .indica* (82.33%) were significantly reduced leaf infections (leaf spot)(Table-6).This is consistent with the earlier reports that many plant products contain fungi-toxic constituents that have the potential to control plant diseases [15,16,17]. Coincidentally plant extracts increased all growth parameters including yield along with reduction of plant diseases. Extracts of *P.nigrum* and *A. marmelos* increased the number of fruits in eggplants. Overall this study reveals the potential of *P.nigrum, A.marmelos, A.conyzoides, and A.indica* extracts to control the wilt and leaf spot disease of brinjal. Further studies on different solvent extract, active ingredients of plants and their mode of action are necessary.

CONCLUSION

The result of present study can be further exploited for formulating integrated disease management schedule of brinjal wilt and leaf spot. More investigations are

needed to investigate this regard for isolation and characterization of antifungal moieties and recommendations in field applications.

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